**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY**
**DEPARTMENT OF PESTICIDE REGULATION**
**MEDICAL TOXICOLOGY BRANCH**

# SUMMARY OF TOXICOLOGY DATA

**ACTIVE INGREDIENT:** FLUBENDIAMIDE (NNI-0001)

**Chemical Code #** 5948, **Document Processing Number (DPN) #** 53013
**SB 950 #** N/A
11/02/07

## I. DATA GAP STATUS

| | |
|---|---|
| **Chronic toxicity, rat:** | No data gap, no adverse effect |
| **Chronic toxicity, dog:** | No data gap, no adverse effect |
| **Oncogenicity, rat:** | No data gap, no adverse effect |
| **Oncogenicity, mouse:** | No data gap, no adverse effect |
| **Reproduction, rat:** | No data gap, no adverse effect |
| **Teratology, rat:** | No data gap, no adverse effect |
| **Teratology, rabbit:** | No data gap, no adverse effect |
| **Gene mutation:** | No data gap, no adverse effect |
| **Chromosome effects:** | No data gap, no adverse effect |
| **DNA damage:** | No data gap, no adverse effect |
| **Neurotoxicity:** | Not required at this time |

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Toxicology one-liners are attached.

All record numbers for the above study types through 234182 (vol. no. 53013-0204) were examined.

In the 1-liners below:
** indicates an acceptable study.
**Bold face** indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.

File name: T071102
Revised by Moore, 11/2/07

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

### COMBINED, RAT

NOTE: There are separate studies by the same author for chronic and oncogenicity rat data.

### CHRONIC TOXICITY, RAT

**53013-0167 226599 Enomoto, A., "NNI-0001: Repeated dose 1-year oral toxicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, April 1, 2004. Laboratory Study # IET 01-0079. Twenty-five SPF Fischer344/DuCrj rats/sex/group were dosed in diet with Flubendiamide (NNI-0001), Lot No. 1FH0018P, 97.8% purity, for 1 year in a standard chronic toxicity study. Achieved dose levels in treated males were 0.8, 2.0, 79, and 822 mg/kg/day in low to high dose groups, respectively. Corresponding achieved dose levels in females were 1.0, 2.4, 98, and 998 mg/kg/day, respectively. NOEL = 50 ppm in both sexes. Liver was a major target organ, with periportal hepatocytic fatty change and diffuse hepatocytic hypertrophy observed in nearly all 2000 and 20000 ppm females, but not in males at any dose level. Liver pathology was grossly evident as enlarged or "dark in color" (also observed in most 2000 ppm females and in nearly all 20000 ppm females, but neither finding was observed in males). Liver weights were significantly elevated in both sexes at 2000 and 20000 ppm, with females showing the largest relative weight effects. Elevated γ-GTP in 2000 ppm and 20000 ppm females (no clear response in males) was probably associated with liver pathology. Thyroid follicular cell hypertrophy was observed in the majority of 2000 ppm males and females, and in all 20000 ppm rats on study. Hematology showed a moderate but consistent reduction in hematocrit and in hemoglobin concentration in 20000 ppm males and in 2000 and 20000 ppm females. Derived parameters MCV and MCH were also depressed in these groups. Prothrombin Time (PT) and Activated Partial Prothrombin Time (APTT), measured only at Week 52, suggested a treatment-related deficiency in the two highest dose groups of males, but there was no coherent treatment effect in females. Study is acceptable, with no adverse effects. Aldous, 10/4/07.

### CHRONIC TOXICITY, DOG

**53013-0165 226600 Kuwahara, M., "NNI-0001: 52-week chronic toxicity study in dogs," The Institute of Environmental Toxicology, Ibaraki, Japan, 4/16/04. Laboratory Study #: IET 02-0035. Four beagles per sex per group were dosed in diet with Flubendiamide (NNI-0001), 96.7% purity, at 0, 100, 1500, or 20000 ppm. Achieved dose levels in respective treated groups were 2.2, 35, and 484 mg/kg/day for males and 2.5, 38, and 533 mg/kg/day for females. NOEL = 100 ppm, with liver as the chief target organ. There were significantly elevated liver weights in 1500 males and in 20000 ppm males and females. Alkaline phosphatase was markedly elevated in treatment-related fashion in 1500 and 20000 ppm males and females (without specific indications of biliary impairment). There was brown pigment deposition associated with Kupffer cells as a microscopic response at 20000 ppm (2 males and 1 female affected). Plasma ALT was significantly elevated in 20000 ppm males and females. Apparently unrelated to liver toxicity was a hematology sign of reduced APTT: statistically significant throughout the study in 1500 ppm females and in 20000 ppm males and females (without primary clinical significance). Body weights appeared to be reduced by treatment in 1500 ppm males and in 20000 ppm males and females, although differences were not statistically significant. Possible effects on food consumption was not detected, since daily rations of 250 g powdered diets were fully consumed by all dogs. Study is acceptable, with no adverse effects. Aldous, 10/10/07.

## ONCOGENICITY, RAT

**53013-0169 226601 Enomoto, A., "NNI-0001: Carcinogenicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 4/30/04. Laboratory Study # IET 01-0080. Fifty SPF Fischer344/DuCrj rats/sex/group were dosed in diet with Flubendiamide (NNI-0001), predominantly from a lot of 97.8% purity, for 2 years at 0, 50, 1000, or 20000 ppm. Achieved dose levels in treated groups were 1.70, 34, and 705 mg/kg/day in low to high group males, and 2.15, 44, and 912 mg/kg/day in corresponding females. NOEL (M/F) = 50 ppm (1.7 mg/kg/day in males, 2.15 mg/kg/day in females). Marked effects at 1000 and 20000 ppm in both sexes included hepatocytic periportal fatty change and increased incidence of chronic progressive nephropathy. Liver weights were significantly elevated at these dose levels in both sexes. Histopathology findings at these two dose levels specific to females included diffuse hepatocyte fatty change, diffuse hepatocytic hypertrophy, thyroid follicular cell hypertrophy, and folliculitis of dorsal skin. Relative kidney weights were elevated in 1000 and 20000 ppm females. Thyroid follicular cell hypertrophy was not increased in males at 1000 ppm, but was present in most males at 20000 ppm. Study is acceptable, with no adverse effects. Aldous, Oct. 4, 2007.

## ONCOGENICITY, MOUSE

**53013-0170 226602 Takeuchi, Y., "NNI-0001: Carcinogenicity study in mice," The Institute of Environmental Toxicology, Ibaraki, Japan, May 6, 2004. Laboratory Study # IET 01-0126. Groups of 52 (SPF) ICR (Crj:CD-1) mice were dosed in diet with Flubendiamide (NNI-0001), 96.7% purity, for 78 weeks in a standard oncogenicity study at 0, 50, 1000, or 10000 ppm. Achieved dose levels in treated males were 4.8, 94, and 988 mg/kg/day in treated males, and 4.4, 93, and 937 mg/kg/day in treated females. NOEL = 50 ppm (M: 4.8 mg/kg/day, F: 4.4 mg/kg/day), based on hepatocellular fatty change and centrilobular hypertrophy in both sexes, and on thyroid follicular cell hypertrophy with hydropic change and an increase in large-sized follicles in both sexes at 1000 and 10000 ppm. Additional thyroid effects at 10000 ppm included altered colloid in thyroids of males and females, and a slight increase in follicular cell hyperplasia in 10000 females. Acceptable, with no adverse effects. There was no treatment-related oncogenicity. Aldous, 10/9/07.

## REPRODUCTION, RAT

**53013-0166 226598 Hojo, H., "NNI-0001: Reproductive toxicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 5/17/04. Laboratory Study # IET 01-0127. Twenty-four Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats/sex/group were dosed in diet with Flubendiamide (NNI-0001) (96.7% purity), Lot No. 1FH0019M for two generations, with continuous treatment through parturition of their respective mates (males) or through lactation (females) in a standard reproduction study at 0, 20, 50, 2000, or 20000 ppm. Achieved pre-mating dose levels were 1.3, 3.3, 131, and 1307 mg/kg/day in treated F0 males, 1.6, 4.0, 162, and 1636 mg/kg/day in treated F0 females, 1.6, 4.0, 159, and 1577 mg/kg/day in treated F1 males, and 1.8, 4.6, 176, and 1808 mg/kg/day in treated F1 females. This was a standard 2-generation reproduction study, including evaluations of estrous state prior to mating, onsets of pup developmental features of preputial separation and vaginal opening, anogenital distances evaluations in F2 pups at PND 4, sperm head counts from testes, and sperm motility, morphology, and counts were from the epididymides. RESULTS: **Parental systemic toxicity NOEL = 50 ppm.** Findings include liver histopathology, particularly in females, of periportal hepatocellular fatty change, diffuse hepatocellular hypertrophy, and deposition of brown pigment in the portal area. There was thyroid follicular cell hypertrophy in both sexes. Both liver and thyroid histopathology were accompanied by elevated organ weights and darkened gross appearance. Kidney weights were significantly elevated in females of both generations at 2000 to 20000 ppm, and spleen weights were significantly lower than controls in 2000 and 20000 ppm females, in both cases without associated histopathology. Ovarian interstitial cell vacuolation was observed in both generations at 20000 ppm, and in four F0 dams at 2000.

**Parental reproductive effects NOEL = 2000 ppm** (there were several maternal deaths at the time of parturition at 20000 ppm: 3 in the present 2-generation study and 2 in the supplementary 1-generation study reported in Record No. 226621). **Offspring viability and growth NOEL = 50 ppm**. Key findings at 2000 ppm included "enlargement of the eyeball," commonly associated with histopathology of synechia, keratitis, hemorrhage, cataract, and hydropic degeneration of basal layer of corneal epithelium; slight delay in preputial separation; thyroid follicular cell hypertrophy in both sexes; statistically significant liver weight increases in both sexes; general decreases (usually statistically significant) in spleen and thymus weights in both sexes; and liver and thyroid histopathology in weanlings of both sexes that was similar to that of parental rats. The following additional observations were limited to 20000 ppm: pup body weight decrement late in the lactation period (4-5 g), and equivocal indications of increased early neonatal deaths. Study is acceptable, with no adverse effects. Aldous, 10/25/07. [See also the following record.]

53013-0189 226621 Hojo, H., "NNI-0001: One-generation reproductive toxicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 5/17/04. Laboratory Study # IET 03-0013. Twenty-four Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats/sex/group were dosed in diet with Flubendiamide (NNI-0001) (96.7% purity), Lot No. 1FH0019M for one generation, with continuous treatment through parturition of their respective mates (males) or through lactation (females) in a follow-up reproduction study at 0, 50, 200, 2000, or 20000 ppm. Achieved pre-mating dose levels were 3.2, 13, 127, and 1287 mg/kg/day in treated F0 males, 3.8, 15, 149, and 1490 mg/kg/day in treated F0 females. Selected F1 pups (typically 1/sex/litter) were maintained long enough to evaluate sexual maturation benchmarks of vaginal opening or preputial separation. Histopathology to the eyes from the present study was reported separately as DPR Document No. 53013-0188, Record No. 226620, Laboratory Study No. IET 04-0075. Those results are included in this review. Combining results of this supplementary study with the main reproductive study, NOEL's for some offspring effects are reduced from 2000 ppm to 200 ppm, as follows. **Parental Systemic Effects**: The supplementary study does not alter the overall NOEL of 50 ppm for parental systemic effects, since this study did not evaluate adult rat histopathology. Incidences of enlarged eyeballs in post-weaning rats are suggestive of a treatment effect at 20000 ppm, but not in lower groups (equivocal but plausible, and not statistically significant). **Parental Reproductive Effects:** The two studies together confirm a parental reproductive toxicity NOEL = 2000 ppm, based on maternal mortalities at the time of parturition at 20000 ppm. **Offspring Viability and Growth Effects:** There is no change in the original NOEL for this category (50 ppm), primarily because there are no data for histopathology of F1 rats in the intermediate groups in the supplementary study. For parameters other than histopathology, the NOEL is 200 ppm, i.e. none of the re-examined parameters had reproducible responses below 2000 ppm. Key tissues were preserved in formalin. If these tissues were to be examined for histopathology, it would be possible to determine whether there is histopathology in offspring at 200 ppm, and thus whether the overall NOEL for this category should be changed. No adverse effects. Aldous, valid supplementary data, 10/22/07.

53013-0188 226620, Y. Takeuchi, "NNI-0001: One-generation reproductive toxicity study in rats: histopathological examination of the eyes of weanlings," June 8, 2005, Laboratory Study No. IET 04-0075. This is the eye histopathology segment of 53013-0189 226621, 5/17/04, Laboratory Study # IET 03-0013. DPR examination of this record is included in the review of Record No. 226621 (see above paragraph).

## TERATOLOGY, RAT

**53013-0165 226597 Aoyama, H., "NNI-0001: Teratogenicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 12/17/03. Laboratory Study # IET 02-0036. Twenty-four Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) dams/group were dosed by gavage with

Flubendiamide (NNI-0001), 96.7% purity at 0, 10, 100, or 1000 mg/kg/day in aqueous 1% CMC suspension during gestation days 6-19 in a standard developmental toxicity study. Maternal NOEL = 10 mg/kg/day (dose-related increases in liver weights). Developmental toxicity NOEL = 1000 mg/kg/day (no developmental toxicity was observed at any dose level). Study is acceptable, with no adverse effects. Aldous, 10/10/07.

53013-0191 226623 Aoyama, H., "NNI-0001: Preliminary teratogenicity study in rats," The Institute of Environmental Toxicology, Ibaraki, Japan, 12/19/02. Laboratory Study # IET 01-0113. Seven Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) dams/group were dosed by gavage with Flubendiamide (NNI-0001), 98.5% purity at 0, 20, 100, or 1000 mg/kg/day in a pilot developmental toxicity study. This pilot study determined that dose levels up to 1000 mg/kg/day could be tolerated in the definitive study. Findings suggestive of possible treatment effects in this pilot study were not repeated in the definitive study, hence no adverse effects are indicated. No DPR worksheet for this pilot study is necessary. Aldous, 8/29/07.

## TERATOLOGY, RABBIT

**53013-0164 226596 Takahashi, K., "NNI-0001: Teratogenicity study in rabbits," The Institute of Environmental Toxicology, Ibaraki, Japan, Dec. 5, 2002. Laboratory Study # IET 01-0128. Groups of 25 Japanese White (Kbl:JW) rabbits were dosed by gavage (1% CMC suspension) with 0, 20, 100, or 1000 mg/kg/day Flubendiamide (NNI-0001), Lot # 1FH0019M, 96.7% purity, on gestation days 6-27 in a standard developmental toxicity study. Maternal NOEL = 100 mg/kg/day (loose stools). Developmental NOEL = 1000 mg/kg/day (no developmental effects). Study is acceptable, with no adverse effects. Aldous, Oct. 12, 2007.

53013-0190 226622 Takahashi, K., "NNI-0001: Preliminary teratogenicity study in rabbits," The Institute of Environmental Toxicology, Ibaraki, Japan, 9/21/02. Laboratory Study # IET 01-0030. Groups of six Japanese White (Kbl:JW) rabbits were dosed by gavage (1% CMC suspension) with 0, 30, 100, 300, or 1000 mg/kg/day Flubendiamide (NNI-0001), 98.5% purity, on gestation days 6-27 in a pilot study. Investigators determined that test article was not toxic in dams nor in fetuses, hence the dose range for the definitive study was justified, with no adverse effects indicated. Aldous, 8/29/07.

## GENE MUTATION

**53013-0171 226603 Inagaki, K., "Bacterial reverse mutation test of NNI-0001," Nihon Nohyaku Co., Ltd., Osaka, Japan, 7/25/03. Laboratory Study # GA-08, 02-0017. Flubendiamide (NNI-0001), Lot No. 1FH0018P, purity 97.8%, was assessed in a pre-incubation reverse mutation test with *S. typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA. The range-finding test assessed levels up to 5000 µg/plate in 4-fold steps (N = 3 was used throughout). In that range-finding study, plates without S-9 had too much precipitation to count any colonies in all 5 strains at 5000 µg/plate, and in two strains at 1250 µg/plate. At 313 µg/plate without S-9, precipitation was sufficient that colonies could not be counted with the colony counter, but it was possible to count colonies with unaided eyes. Thus 313 µg/plate was the selected top dose without S-9 (with lower doses in 3-fold steps, down to 3.86 µg/plate). Plates with S-9 evaluated in the range-finding study could be read for revertant colonies with the unaided eye at 5000 µg/plate, however precipitation was sufficient at that dose that the plates could not be evaluated to assess cytotoxicity. There was precipitation, but no evident cytotoxicity with S-9 at 1250 µg/plate. Investigators chose 5000 µg/plate as the top dose with S-9 (with lower doses in 3-fold steps, down to 61.7 µg/plate). There were no increases in revertants in either of the main tests (with or without S-9). Positive controls were functional. There was some precipitation in all groups without S-9 at the highest dose levels (104 and 313 µg/plate). There was no evident cytotoxicity without S-9. Tests with S-9 found precipitation at 556 µg/plate and above in all groups. At the two highest dose levels with S-9

(1670 and 5000 µg/plate), precipitation was sufficient that investigators could not assess plates for cytotoxicity. Since the range-finding tests also incorporated the same dose ranges, and also had N=3 with and without S-9, investigators considered the range-finding study to constitute an acceptable second negative test. Study is acceptable, with no adverse effects. Aldous, 9/20/07.

**53013-0172 226604 Herbold, B., "NNI-0001 480 SC: Salmonella/microsome test: plate incorporation and preincubation method, " Bayer HealthCare AG, Wuppertal, Germany, 5/19/04. Laboratory Study # T 3073247. *S. typhimurium* strains TA100, TA1535, TA98, TA 102, and TA1537 were subjected to a standard reverse mutation test (including pre-incubation for 20 min at 37°C, and plate incorporation phases). Plates were incubated at 37°C after pouring in both cases. Test article was NNI-0001 480 SC (489.54 g/l flubendiamide). Formulation was applied in triplicate at 0, 16, 50, 158, 500, 1581, and 5000 µg/plate with and without S-9, in both plate incorporation and preincubation tests. All tests were negative, with and without S-9. Positive controls were functional. There was no cytotoxicity nor growth inhibition noted. Results did not address whether or not there was precipitation of test article, however colonies were countable in all treatment groups. Acceptable, with no adverse effects. Aldous, 10/29/07.

**53013-0174 226606 Herbold, B., "NNI-0001: V79/HPRT-test *in vitro* for the detection of induced forward mutations," Bayer HealthCare AG, Wuppertal, Germany, June 12, 2003. Laboratory Study # T 2071518. V79 cells, derived from lung tissue of a male Chinese hamster, were used to assess forward mutation to a 6-thioguanine-tolerant phenotype. Flubendiamide (NNI-0001) (96.6% purity) was evaluated in the range of 7.5 to 240 µg/ml. There were two independent trials with and without S-9. Positive controls, ethylmethanesulfonate (EMS) and dimethylbenzanthracene (DMBA) were effective. Flubendiamide at 240 µg/ml was toxic in all trials with and without S-9 based on reduced relative growth, and precipitated in the medium. Both trials without S-9 were uneventful. Trial 1 with S-9 found comparatively high mutant frequencies in the range of 60 to 120 µg/ml (about 2-3 times the highest concurrent control value). As a result, investigators performed the second trial with S-9 with comparatively closely-spaced treatment levels in the range of possible treatment response. The second trial provided a rather definitive negative response. Concurrent negative and vehicle controls tended to be lower than historical means, which provides additional confidence in considering this test to be negative. The second trial with S-9 found precipitation at 160 through 240 µg/ml. Study is acceptable, with no adverse effects. Aldous, 9/24/07.

## CHROMOSOME EFFECTS

**53013-0175 226607 Miyahana, K., "Micronucleus test of NNI-0001 in mice," Nihon Nohyaku Co., Ltd., Osaka, Japan, Dec. 9, 2003. Laboratory Study # GA-08, 02-0020. Five ICR (Slc:ICR) mice/sex per group were dosed orally either 24 hr or 48 hr before sacrifice with 0, 500, 1000, or 2000 mg/kg of Flubendiamide (NNI-0001), Lot No. 1FH0018P, purity 97.8%, prior to removal of femurs for bone marrow cell collection. Giemsa-stained smears examined: (2000 immature erythrocytes/slide for micronuclei counts). Investigators also estimated the proportion of immature erythrocytes to total erythrocytes. There were no treatment effects on micronuclei counts in either sex or in either pretreatment time interval. The proportion of immature erythrocytes did not vary with treatment. The study is acceptable, with no adverse effects. Aldous, 10/25/07.

**53013-0176 226608 Herbold, B., "NNI-0001: Micronucleus-test on the male mouse," Bayer HealthCare AG, Wuppertal, Germany, Jan. 10, 2005. Laboratory Study # T 0073947. Five male Hsd/Win: NMRI mice/group were dosed ip with two treatments of Flubendiamide (NNI-0001), purity 98.9%, Batch No. 3FH 00 32 M, administered in 0.5% Cremophor, 20 ml/kg b.w. Treatments were 24 hr apart. The second injection was 24 hr prior to sacrifice, at which time

investigators collected and processed femoral marrow cells for the micronucleus test. Positive control was cyclophosphamide, 20 mg/kg ip in the same vehicle, administered only once 24 hr before sacrifice. Investigators reported that flubendiamide groups experienced "apathy, roughened fur, loss of weight, sternal recumbency, spasm and difficulty in breathing," but provided no further information on frequencies or degrees of these observations. No flubendiamide groups had significant increases in PCE's with micronuclei when compared to concurrent controls by Mann-Whitney tests (criteria of $p < 0.05$). All results were within historical control range for the test facility. Positive control micronuclei counts were significantly elevated compared to concurrent controls ($p < 0.01$). Study is classified by DPR as negative and acceptable, with some deficiencies as indicated in the review. Aldous, 9/24/07.

**53013-0173 226605 Miyahana, K., "*In vitro* chromosomal aberration test of NNI-0001 in cultured Chinese hamster cells," Nihon Nohyaku Co., Ltd., Osaka, Japan, March 4, 2003. Laboratory Study # GA-08, 02-0116. Cells from a Chinese hamster lung (CHL) cell line provided by the Japan Health Sciences Foundation were used to assess chromosomal aberrations with flubendiamide (purity 97.8%). A total of 200 metaphases were counted at each dose level in each test. Highest dose levels for the different phases of the study based on cytotoxicity results were 2200 µg/ml for 6 hr exposures with and without S-9 (followed by 14 hr in fresh medium in either case), 1200 µg/ml for continuous exposure without S-9 for 20 hr prior to harvest, and 500 µg/ml for continuous exposure without S-9 for 40 hr prior to harvest. Cytotoxicity studies found precipitation at 550 µg/ml with or without S-9 in the 6 hr trials, and at 300 µg/ml without S-9 in the 20 hr trial, and at 125 µg/ml without S-9 in the 40 hr trial. All chromosomal aberration assays involved 2-fold and 4-fold dilution steps from respective high dose levels. All flubendiamide trials were negative. Positive controls (10 µg/ml cyclophosphamide with S-9, or 0.07 to 0.10 µg/ml Mitomycin C without S-9) were functional. Study is acceptable, with no adverse effects. Aldous, 10/25/07.

## DNA DAMAGE

Mouse micronucleus studies in the previous section often are applied to DNA Damage data requirements.

## NEUROTOXICITY

**Rat Acute Neurotoxicity Study**

0177, 0204; 226609, 234182; "An Acute Oral Neurotoxicity Screening Study with Technical Grade NNI-001 in Fischer 344 Rats" (Gilmore, R.G., Bayer CropScience LP, Toxicology, Stilwell, KS, Report No 200489, 04/17/03). 870.62. Technical Grade NNI-0001 (Batch No. 1FH0091M, purity = 97.0%), suspended in 0.5% methylcellulose/0.4% Tween 80 in deionized water, was administered as a single gavage dose to 12 Fischer 344 rats per sex per dose at dose levels of 0 (vehicle only), 200, 700, and 2000 mg/kg. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. No effects on body weight were observed. FOB assessments revealed no treatment-related effects on days 0, 7, and 14. Locomotor and motor activity assessment revealed no treatment-related effects on days 0, 7, and 14. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. NOEL (M/F) = 2000 mg/kg (based on no effects at the highest dose tested). **Unacceptable but possibly upgradable** with the submission of more recent positive control data specifically more recent FOB with carbaryl, FOB and neuropathology with acrylamide, and neuropathology with trimethyltin data generated within a few years of the time interval of the study in review. (Corlett and Leung, 08/20/07)

**Rat Developmental Neurotoxicity Study**

**53013-0178 226610** Sheets, L. P., "A developmental neurotoxicity screening study with Technical Grade NNI-0001 in Wistar rats," Bayer CropScience LP, Stilwell, KS, 2/17/06.

Laboratory Study # 04-D72-VK. Bayer CropScience Report No. 201448. Thirty mated female Wistar Han CRL:WI (GLX/BRL/HAN) IGSBR rats/sex/group were placed on a standard developmental neurotoxicity study for treatment with Flubendiamide (NNI-0001), 97.3% purity, from gestation day 6 through lactation day 21 at 0, 120, 1200, or 12000 ppm, targeting 0, 10, 100, and 1000 mg/kg/day flubendiamide. Concentrations in diet were reduced as needed during mid- to late lactation to maintain the above mg/kg/day exposures. Achieved dosing was ± 8% of target during gestation and ±12% of target during lactation. Offspring NOEL = 120 ppm, based on eye responses in two 1200 ppm male pups: exophthalmia in one, and enlarged eyeball and general ocular opacity in another; also on delayed mean onset of preputial separation (3 days delay at 1200 ppm, and 4 days delay at 12000 ppm). Findings in female offspring were limited to 12000 ppm, based on reduced body weight by the end of lactation (observed in both sexes), eye effects including enlarged eyeball, corneal opacity and red eyeball (all observed in both sexes), and a delay in vaginal opening of 3 days. Since females appeared to have greater eye responses than males at 12000 ppm, it is prudent to consider 1200 ppm as an effect level for offspring of both sexes. The ocular findings are considered "possible adverse effects." Optic nerve atrophy was slightly elevated in 12000 ppm terminal F1 rats, but was not evaluated at the next lower dose level. This study is unacceptable, but upgradeable upon receipt of two validation studies requested in the discussion section of the DPR review, and upon receipt of histopathology data for 1200 ppm terminal F1 rats. Aldous, 10/19/07.

## METABOLISM

53013-0179 226611 Motoba, K., "Absorption, distribution, metabolism and excretion of radiolabeled NNI-0001 following a single oral administration to male and female rats," Nihon Nohyaku Co., Ltd., Osaka, Japan, 9/16/05. Laboratory Study #: GB-01, 03-0022. Typically sets of 4 fasted rats/sex were dosed once by gavage with Flubendiamide (NNI-0001), $^{14}$C-labeled on either the phthalic acid ring or (to a limited extent) on the aniline ring, radiochemical content after purification > 99.5%, in an aqueous suspension of 1% sodium CMC containing 0.2% Tween 80. This study assessed ADME features including identification of major metabolites following treatment with either 2 or 200 mg/kg flubendiamide. There was no significant excretion of label into expired air. Over 90% of administered dose was excreted in feces regardless of dose or sex. Although urine was a minor route (< 2% of administered dose), males consistently passed more label in urine than did females. High dose levels led to markedly reduced absorption. There was no obvious difference in excretion patterns and there were minimal differences in metabolite chromatograms between the two labeled forms, indicating that there was very little cleavage dividing major portions of the molecule. $T_{max}$ was 6 to 12 hours for blood and plasma. Tissue distribution of label was preferentially to the g.i. tract, liver and other highly perfused organs, and body fat. Low dose treatments found peak tissue levels in males several-fold higher than females. Tissue levels in males were lower by about 90% from 9 hrs to 24 hrs, and by another 90% from 24 hrs to 168 hrs. Females did not clear tissues as rapidly as males, however, so females had higher residual tissue concentrations at 168 hours than males. The primary substituent available for oxidation on flubendiamide is the methyl group on the aniline ring. The most common products were the benzyl alcohol (designated A-16) and the benzoic acid (designated A-18) of that methyl carbon. The less abundant intermediate benzaldehyde was designated as A-17. Substantial sex differences emerged in metabolic profiles. In the feces, the benzyl alcohol was the dominant metabolite in 2 mg/kg males (31-37% of administered dose), followed by parent flubendiamide (15-30% of administered dose), and the benzoic acid metabolite (15-16% of administered dose). In feces of 2 mg/kg females, 66% of administered dose was flubendiamide, 5-6% of administered dose was benzyl alcohol, and there was very little benzoic acid metabolite. There were no other major metabolites. Parent flubendiamide was the predominant labeled constituent in feces of both sexes at 200 mg/kg, indicating saturable absorption. Female rats were very slow to clear

radioactivity: contrast liver label content in 2 mg/kg males dropping from 5.628% to 0.104% of administered dose at 9 hrs and 168 hrs after dosing vs. content in females of 1.623% and 1.305% of administered dose at the same sampling times. Female rats have limited hepatic metabolic capacity for flubendiamide, hence there was appreciable accumulation of parent flubendiamide in the livers of females at 24 hours (an order of magnitude more than all extractable metabolites combined). In males, very little parent flubendiamide was found in liver (less than half of the percent of dose represented by benzyl alcohol or benzaldehyde metabolites). Liver in males had smaller but measurable amounts of the sole identified product of cleavage between the two ring structures of flubendiamide (the aniline fragment). This is a useful component of the rat metabolism series. Aldous, 10/30/07.

53013-0180 226612 Motoba, K., "Absorption, distribution, metabolism and excretion of [phthalic ring (U)-$^{14}$C] NNI-0001 following 14 repetitive oral administration to male and female rats," Nihon Nohyaku Co., Ltd., Osaka, Japan, 9/16/05. Laboratory Study #: GB-01, 03-0222. Typically sets of 4 non-fasted rats/sex were gavage-dosed daily for 14 days with Flubendiamide (NNI-0001), $^{14}$C-labeled on the phthalic acid ring, radiochemical purity after purification of 99.2%, in an aqueous suspension of 2% sodium CMC containing 0.4% Tween 80. This study assessed ADME features including quantification of major metabolites. Rats were sacrificed either 9 hr, 24 hr, or 168 hr after the final (14$^{th}$) dosing. Results from the present study should be compared to the primary single dose study (DPR Document No. 53013-0179, Record No. 226611). There was no substantial change in blood or plasma levels when comparing rats sampled 24 hr after 1, 6, or 13 daily doses. Distribution to tissues between 9 hr and 168 hr after repetitive dosing for 14 days found that label was moving through the g.i. tract more slowly in the present study than in the single-dose study during the first 24 hr. At 24 hours in the present study, much more of the label remained in the intestinal contents than was the case in the single-dose study. Also, tissue levels were not decreasing as rapidly in this study from 9 hr to 24 hr after dosing compared to the single dose study. By 7 days after dosing, tissue levels in the two studies were comparable. Overall absorption in the present study appeared to be markedly lower than was observed in the single dose study, based on percent of administered label found in the urine. This appears to reflect comparatively low absorption of a dose administered to non-fasted animals. Females showed less metabolized label and greater long-term tissue retention of label than males, consistent with other studies. Parent flubendiamide comprised 82% and 91% of fecal metabolites in males and females, respectively. Relative proportions of remaining fecal label consisted of the benzyl alcohol metabolite (A-16) (7.2% of excreted radioactivity in males, and 2.2% in females), the benzoic acid metabolite (A-18) (2.8% of excreted radioactivity in males and below detection in females), with other metabolites uncharacterized or in very small amounts. This study reported quantifiable amounts of the iodophthalimide metabolite (A-14) of flubendiamide in fat. This metabolite, involving cleavage of all components distal to the phthalamide nitrogen, had not been found in measurable amounts in excreta. This study is a useful component of the rat metabolism series. Aldous, 10/30/07.

53013-0181 226613 Motoba, K., "Biliary excretion study of [phthalic ring-(U)-$^{14}$C] NNI-0001 following a single oral administration to male and female rats," Nihon Nohyaku Co., Ltd., Osaka, Japan, 5/14/04. Laboratory Study # GB-01, 01-0151. Flubendiamide (purified to 99.6% purity for non-labeled a.i. and for $^{14}$C-labeled on the phthalic acid ring) was administered once by gavage (aqueous suspension of 2% sodium CMC containing 0.4% Tween 80) to F-344 (F344/DuCrj) rats provided with biliary cannulae. Three males and 6 females received 2 mg/kg in the definitive study. [Additional rats were dosed twice at 16-hr intervals with 20 mg/kg a.i. to facilitate characterization of metabolites.] Investigators determined the excretion of label in bile, urine, feces over 48 hours, and determined the residues in g.i. tract contents, in the rinsed g.i. tract, the liver, and in residual carcass. Investigators also identified the major metabolites in bile, feces, and g.i. tract contents at 48 hours. Absorption was slow: contents of the g.i. tract at

48 hours contained 60% and 51% of administered label in males and females, respectively. Bile contained 11.1% and 3.3% of administered label in males and females, respectively. Feces in these cannulated rats contained 12.8% and 11.0% of administered label in males and females. Urine contained only 0.75% and 0.15% of administered label in males and females: thus urinary metabolites were not assessed in this study. Parent flubendiamide was the dominant component of [feces plus g.i. content] in both sexes (94% of label recovered from these sources in males, and 99% in females). The only quantifiable metabolite observed in feces or g.i. content was the benzyl alcohol metabolite, possibly resulting from intestinal microbial action of non-absorbed material. Much of the biliary profile in males consisted of oxidation products of the methyl group on the aniline ring, as was noted in Record No. 226611. There was also some oxidation of the methyl groups between the phthalamide nitrogen and the sulfonyl group (including compounds designated A-19, A-20, A-25, and A-29 by investigators). Most commonly the oxidation products of the methyl group on the aniline ring were not conjugated, however it appears that oxidation of a methyl group between the phthalamide nitrogen and the sulfonyl group frequently was followed by glucuronide conjugation in males. Both sexes were capable of glutathione formation of parent compound, conjugation proposed by investigators to be on the phthalic ring. In females, glutathione conjugates (and downstream derivatives of the conjugation) were the dominant biliary metabolites. A cyclic acetal was observed in bile, particularly in males. This metabolite had not been identified in Record No. 226611. It may have been one of the uncharacterized metabolites in that study, or the acetal may not be stable in the intestinal environment. This is a useful component of the rat metabolism series. Aldous, 10/30/07.

53013-0182 226614 Motoba, K., "*In vitro* metabolism study of NNI-0001," Nihon Nohyaku Co., Ltd., Osaka, Japan, 8/27/04. Laboratory Study # GA-05, 03-0181. This study evaluated abilities of microsomes from various sources to metabolize flubendiamide to the benzyl alcohol metabolite (designated A-16). Rat, dog, mouse, and human microsomes (from both sexes in all species) were all able to metabolize flubendiamide to A-16, except for microsomes from female rats. (Human microsomes, but not rat microsomes, additionally produced small amounts of hydroxybenzoic acid metabolite, A-20, however metabolism to A-16 appears to be a valid indicator of overall flubendiamide metabolism.) Antisera to CYP isoforms tested in male rat microsomes found that anti-CYP3A2 sera had no effect on A-16 production, whereas anti-CYP2C11 markedly inhibited A-16 production. In contrast, recombinant microsomes expressing rat CYP3A2 produced A-16, whereas other isoforms including CYP2C11 recombinant microsomes did not. Antibody effects on human liver microsome activities found that anti-rat CYP3A2 and anti-human CYP3A4 caused similar and substantial inhibition in microsomes from male or female human liver (it was noted that anti-rat CYP3A2 was strongly reactive toward CYP3A4). Of 5 recombinant microsomes expressing human liver P450 isoforms evaluated for A-16 production, only CYP3A4 yielded measurable A-16. Investigators concluded that the lack of metabolic capacity of rat liver toward flubendiamide does not reflect a parallel lack in humans of either sex. This is a useful component of the rat metabolism series. Aldous, 9/19/07.

53013-0183 226615 Motoba, K., "Toxicokinetics of NNI-0001: Concentration in selected organs, tissues and plasma following repetitive daily administration to rats and mice," Nihon Nohyaku Co., Ltd., Osaka, Japan, Dec. 5, 2005. Laboratory Study # GA-25, 05-0230. F-344 (F344/DuCrj) rats and Crlj: CD1® (ICR) mice (4/species/sex/dosing period) were dosed with 200 mg/kg/day unlabeled flubendiamide, purity 96.7% by gavage in corn oil. Treatments were daily for 1, 7, or 14 days. Animals were sacrificed 24 hr after the final dose. Investigators determined concentrations of flubendiamide and of the iodophthalimide metabolite (A-14) in plasma, liver, and fat. Flubendiamide did not increase in the in these tissues over time in males. There was a marginal accumulation in females in all studied tissues, reaching steady state by day 7. A-14 appeared to be accumulating in fat in rats of both sexes, reaching steady

state by day 7. Flubendiamide was always more concentrated in liver and fat than in plasma, and concentrations were much higher in females than in males. Day 7 concentrations of flubendiamide in liver and fat of male rats at day 7 were 1.3 and 8.9 mg/kg, respectively. Liver and fat concentrations in female rats were 26.7 and 68.0 mg/kg. A-14 concentrations in male and female rats for liver and fat at day 7 were identical at < 1.0 and 2.9 mg/kg, respectively. Flubendiamide concentrations on day 7 in liver and fat of male mice were 2.3 and 3.4 mg/kg, respectively. Corresponding levels in female mice were 2.4 and 1.9 mg/kg (i.e. much unlike the marked accumulation in female rats). A-14 levels were generally below quantification limits in both male and female mice. This study indicates that limited metabolic capacity of female rats toward flubendiamide and tendency to metabolize significant amounts of A-14 do not apply to either sex of mice. This is a useful component of the rat metabolism series. Aldous, 9/19/07.

NOTE: The collective metabolism data in the above several records address the data requirements for this study type. Aldous, Oct. 3, 2007.

## MECHANISTIC STUDIES

53013-0187 226619 Freyberger, A., "Studies on interactions with Iodothyronine Deiodinase Type I *in vitro*," Bayer AG Institute of Toxicology, Wuppertal, Germany, 6/20/03. Study No. T2071996. This study sought to determine whether flubendiamide inhibited this key enzyme, which deiodinates $T_4$ to $T_3$ (the ultimate hormone). Investigators used the 10000 x g supernatant of rat liver homogenate as an enzyme source and labeled $rT_3$, i.e. [5'$^{125}$I]-3,3'-triiodothyronine, as the substrate. Reaction was assayed by isolating and counting free $^{125}$I iodide after removal of labeled organics by an SPE column (RP 8). Positive controls were propylthiouracil (PTU) and erythrosin B. The former was an effective inhibitor, however the latter gave inconsistent results under study conditions, possibly because of inefficient removal of labeled $rT_3$ by the SPE column. Flubendiamide did not inhibit the deiodinase at concentrations up to 100 µM, considered to be a practical limit for this assay. Investigators thus concluded that observed effects of flubendiamide on thyroids were unlikely due to inhibiting this enzyme. Useful supplementary data. No DPR worksheet. Aldous, 8/29/07.

53013-0185 226617 Amanuma, T., "Effect of NNI-0001 administration on the thyroid-related hormones and liver drug-metabolizing enzymes in female F-344 rats," Product Safety & Pharmaceutical Research Unit, Osaka, Japan, 6/29/05. Laboratory Study # GA-11, 02-0162. Ten females/dose/group were dosed in diet for either 7 days or 28 days with Flubendiamide (NNI-0001), purity 96.7% in this supplementary dietary study. Investigators assessed effects on thyroid, liver, and pituitary, including these organ weights, thyroid-associated hormones ($T_3$, $T_4$, and TSH), liver microsomal enzyme activities, and gross and microscopic pathology. Liver weights were significantly elevated at both dose levels by day 7 and day 28. Livers displayed increased cytochrome P-450 content, and elevated UDP-glucuronyl transferase and ethoxyresorufin O-dealkylase activities. Hepatocyte hypertrophy and periportal vacuolation were significantly elevated at 10000 ppm at 7 days, and at both 1000 and 10000 ppm at 28 days. The hypertrophy increased in degree over this time period. Mitotic figures in hepatocytes were common at both dose levels at 7 days, but not observed at 28 days. Thyroid changes appeared to progress slowly compared to the liver: thyroid weights were not affected at 1 week in either group, but were elevated (dose-related) in both groups at 4 weeks. Of thyroid-related hormones, there was a consistent elevation in $T_3$ concentration in sera of 1000 and 10000 ppm groups, emerging after day 3 but before day 7. There was no apparent change in $T_4$ levels. There were meaningful increases in TSH at 28 days but not at day 14 or before: values were over twice pre-study concentrations at 28 days. Thyroid follicular cell hypertrophy was observed by day 7, however there was an increase in degree of response at day 28. There were no demonstrated effects on the pituitary. These data support the concept that liver enzyme activity induction is a major factor in increasing thyroid function through enhanced metabolism of

hormones such as $T_4$ and associated feedback response: useful supplementary data, Aldous, 10/30/07.

53013-0186 226618 Langewische, F. W., "NNI-0001: Perinatal ocular toxicity study in CD-1 mice following exposure via diet," Bayer HealthCare AG, Wuppertal, Germany, 1/20/06. Laboratory Study #: AT02781. This test may have been conducted because "enlargement of the eyeball" was a possible treatment effect in very high dose exposures to rats (see rat reproduction study in the flubendiamide Summary of Toxicology Data). In the present study, groups of 25 inseminated mice/group were dosed in diet with targeted dietary dose levels of 0 or 1000 mg/kg/day flubendiamide from gestation day 6 through lactation day 21. Pups were weaned at lactation day 21, and were maintained off treatment until sacrifice at about 42 days of age. Several commonly-assessed indicators of possible maternal and pup toxicity were assessed throughout the study. There was no evident maternal toxicity. There was a marginal decrement in weanling pup weight in treated pups (12.95 g, vs. 13.66 g in controls, significant $p < 0.05$). There were no other apparent treatment effects on offspring. In particular, pups were examined for possible ocular changes as a part of clinical observations, and no eye effects nor any other effects were found. The study was terminated without further examinations of offspring (i.e. no ophthalmolscopy and no histopathology). Useful supplementary data. Aldous, 10/26/07.

## SUBCHRONIC TOXICITY STUDIES

**Rat Subchronic Dietary Toxicity Study**

0160, 226592; "NNI-0001: Repeated Dose 90-Day Oral Toxicity Study in Rats" (Enomoto, A., The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID IET 01-0013, 02/18/03). 870.31. NNI-0001 (Lot No. 0FH0009P, purity = 97.7%) was admixed to the diet and fed continuously to 10 Fischer (F344/DuCrj) rats per sex per dose at dose levels of 0, 20, 50, 200, 2000, or 20000 ppm (0, 1.15, 2.85, 11.4, 116, and 1192 mg/kg/day, respectively for males and 0, 1.30, 3.29, 13.1, 128, and 1320 mg/kg/day, respectively for females) for 90 days [with 10 additional rats per sex per dose level at 0 and 20000 ppm dose levels to assess reversibility (4-week recovery period used)]. No mortalities occurred. Cageside observations, detailed clinical observations, and functional observations revealed no treatment-related or toxicologically significant effects. Examination of body weight and food consumption data revealed no treatment-related effects. A treatment-related increase in mean platelet count level was observed in main group animals of both sexes at 2000 and 20000 ppm and this increase persisted in recovery group animals. Treatment-related decreases in mean corpuscular volume (in males at 20000 ppm and in females at 200, 2000, and 20000 ppm) and mean hemoglobin and mean hematocrit levels (in females at 2000 and 20000 ppm) were observed in main group animals and these decreases persisted in recovery group animals. A treatment-related increase in the mean total protein level was observed in both sexes at 20000 ppm and this increase persisted in recovery group animals of both sexes. A treatment-related increase in mean relative liver weight was observed in males at 20000 ppm and in females at 200, 2000, and 20000 ppm and persisted in recovery group animals of both sexes. Macroscopic examination revealed treatment-related enlarged liver in males at 20000 ppm and in females at 2000 and 20000 ppm and/or treated-related dark colored liver in both sexes at 20000 ppm; these abnormalities were not observed in recovery group animals. Microscopic examination revealed treatment-related periportal fatty change of the hepatocytes (in males at 20000 and in females at 200, 2000, and 20000 ppm), hypertrophy of the hepatocytes (in females at 2000 and 20000 ppm), and follicular cell hypertrophy in the thyroid (in males at 20000 and in females at 200, 2000, and 20000 ppm); none of these changes were observed in the recovery group animals except for periportal fatty change of the hepatocytes in females and follicular cell hypertrophy in the thyroid in males. **No adverse effects.** NOEL (M) = 11.4 mg/kg/day (200 ppm) and NOEL

(F) = 3.29 mg/kg/day (50 ppm) based on increased mean platelet counts, mean relative liver weights and histopathological changes in the liver and thyroid. **Acceptable**. (Corlett, 05/18/07)

**Mouse Subchronic Dietary Toxicity Study**

0161, 226593; "NNI-0001: Repeated Dose 90-Day Oral Toxicity Study in Mice" (Takeuchi, Y., The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID IET 01-0049, 04/17/02). 870.31. NNI-0001 (Lot No. 0FH0010P, purity = 98.5%) was admixed to the diet and fed to 10 ICR (Crj:CD-1) mice per sex per dose at dose levels of 0, 50, 100, 1000, or 10000 ppm (0, 6.01, 11.9, 123, and 1214 mg/kg/day, respectively for males and 0, 7.13, 14.7, 145, and 1424 mg/kg/day, respectively for females) continuously for 90 days. No mortalities occurred. General clinical observations revealed no treatment-related clinical signs. Examination of body weight and food consumption data revealed no treatment-related effects. Hematological investigations revealed no treatment-related effects. A treatment-related increase in the mean total bilirubin level was observed in females at 10000 ppm. A treatment-related increase in mean relative liver weight was observed in males at 10000 ppm and in females at 1000 and 10000 ppm. Macroscopic examination revealed treatment-related dark colored liver in males at 10000 ppm. Microscopic examination revealed treatment-related centrilobular fatty change of the hepatocytes and hypertrophy of the hepatocytes in both sexes at 1000 and 10000 ppm. **No adverse effects.** NOEL (M) = 11.9 mg/kg/day (100 ppm) and NOEL (F) = 14.7 mg/kg/day (100 ppm) based on increased mean relative liver weights and histopathological changes in the liver. **Supplemental study** (required ophthalmological examinations were not conducted on the eyes of the test animals). (Corlett, 05/24/07)

**Dog 28-Day Dietary Toxicity Study**

0192; 226624; "NNI-0001: Repeated Dose 28-Day Oral Toxicity Study in Dogs" (Kuwahara, M., The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID IET 01-0019, 12/11/01). NNI-0001 (Lot No. 0FH0010P, purity = 98.5%) was incorporated into the basal diet and administered continuously to 1 beagle dog per sex per dose at dose levels of 0 (basal diet only), 40, 400, 4000, or 40000 ppm (0, 1.12, 10.7, 101.1, and 1111 mg/kg/day, respectively, for males and 0, 1.10, 12.0, 120, and 1180 mg/kg/day, respectively, for females) for 28 days. No mortalities occurred. No effects on body weight and food consumption were observed. Loose stool was observed for several days in the male and female at 40000 ppm but was also observed in the untreated female. Hematology and urinalysis revealed no treatment-related effects. Elevated serum alkaline phosphatase levels (when compared with pre-treatment values) were observed in both sexes at 400 ppm and above. Increased absolute and relative liver weights were observed in both sexes at 40000 ppm. Necropsy revealed no gross abnormalities. Histopathological examination of the liver, kidneys, and adrenals revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 1.12 mg/kg/day (40 ppm) and NOEL (F) = 1.10 mg/kg/day (40 ppm) based on an increase in alkaline phosphatase activity. **Supplemental study** (only 1 animal per sex per dose level was used and the test animals were treated for only 28 days). (Corlett, 09/05/07)

**Dog Subchronic Dietary Toxicity Study**

0162, 226594; "NNI-0001: Repeated Dose 90-Day Oral Toxicity Study in Dogs" (Kuwahara, M., The Institute of Environmental Toxicology, Ibaraki, Japan, Laboratory Project ID IET 01-0062, 02/03/03). 870.3150. NNI-0001 (Lot No. 0FH0010P, purity = 98.5%) was admixed to the diet and fed to 4 beagle dogs per sex per dose at dose levels of 0, 100, 2000, or 40000 ppm (0, 2.58, 52.7, and 1076 mg/kg/day, respectively for males and 0, 2.82, 59.7, and 1135 mg/kg/day, respectively for females) continuously for 90 days. No mortalities occurred. Treatment-related loose stool was observed in both sexes at 40000 ppm. Examination of body weight and food consumption data revealed no treatment-related effects. Urinalysis revealed no dose-related effects. A treatment-related decrease in mean activated partial thromboplastin time was

observed at weeks 4, 8, and 13 in both sexes at 2000 and 40000 ppm. A treatment-related increase in the mean total alkaline phosphatase level was observed at weeks 4, 8, and 13 in males at 40000 ppm and in females at 2000 and 40000 ppm. Also, a treatment-related decrease in mean cholesterol levels in males at 40000 ppm at weeks 4 and 8 and a treatment-related increase in mean triglyceride levels in females at 2000 and 40000 ppm at weeks 4, 8, and 13 were observed. A trend toward increased mean relative adrenal weights was observed in both sexes at 40000. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination revealed treatment-related hypertrophy of the cortical cells of adrenals in males at 40000 and in females at 2000 and 40000 ppm. **No adverse effects.** NOEL (M) = 2.58 mg/kg/day (100 ppm) and NOEL (F) = 2.82 mg/kg/day (100 ppm) based on an increase in alkaline phosphatase activity, decreased activated partial thromboplastin time, a trend toward increased mean relative adrenal weights, and hypertrophy of the cortical cells of adrenals. **Acceptable** (Corlett, 06/08/07)

**Rat Repeated Dosing 4-Week Dermal Toxicity Study**

0163, 226595; "Project: NNI-0001 30-Day Toxicity Study in the Rat by Dermal Administration" (Krötlinger, F., Bayer HealthCare AG, PH-PD Toxicology International, Wuppertal, Germany, Study No. T6073709, 12/10/04). 870.3200. NNI-0001 (Batch No. 1FH0019M, purity = 97.1%) was applied to the shaved dorsal skin of 10 Fischer (F344/NHsd) rats per sex per dose at dose levels of 0 (tap water), 10, 100, or 1000 mg/kg/day for 6 hours per day 5 days per week for 4 weeks (7 days per week during the 4$^{th}$ week). No treatment-related mortalities occurred. No clinical signs were observed. Open field observations revealed no abnormalities. Examination of body weight and food consumption data revealed no treatment-related effects. Hematological data revealed no clear dose-response relationship. Serum chemistry data revealed no biologically significant changes. Urinalysis revealed no treatment-related effects. A treatment-related increase in mean relative liver weight was observed in both sexes at 1000 mg/kg/day. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination revealed a treatment-related elevated fat positive reaction (in incidence in females and in severity in males and females) in the periportal zone of the liver at 1000 mg/kg/day. Also, a treatment-related higher incidence of follicular cell hypertrophy in the thyroid was observed in females at 1000 mg/kg/day. **No adverse effects.** NOEL (M/F, systemic) = 100 mg/kg/day (based on increased mean relative liver weights and histopathological changes in the liver and thyroid). NOEL (M/F, skin effects) = 1000 mg/kg/day (based on no effects at the highest dose tested). **Unacceptable but possibly upgradable** with submission of data ensuring that the protocol used sufficiently moistened of the test article. (Corlett, 06/18/07)

**Rat Immunotoxicity Study**

0193, 226625; "Project: NNI-0001 Immunotoxicity Study in Rats- Plaque Assay (4 Weeks Administration by Diet)" (Krötlinger, F. and Vohr, H.-W., Bayer HealthCare AG, PH-R&D Toxicology International, Wuppertal, Germany, Study No. T1073902, 06/13/05). 870.78. NNI-0001 (Batch No. 1FH0019M, purity = 97.1%) was admixed to the diet and fed continuously to 10 Wistar (HsdCpb:WU) rats per sex per dose at dose levels of 0, 40, 400, or 4000 ppm (0, 3.34, 33.60, and 336.31 mg/kg/day, respectively for males and 0, 4.00, 38.35, and 358.84 mg/kg/day, respectively for females) for 4 weeks. No mortalities occurred. No treatment-related clinical signs were observed. Examination of body weight data revealed no treatment-related effects. Hematological data revealed treatment-related decreases in mean hemoglobin and hematocrit levels in females at 400 and 4000 ppm and in mean red blood cell count in females at 4000 ppm. A treatment-related increase in mean relative liver weight was observed in both sexes at 4000 ppm and in females at 400 ppm. Macroscopic examination revealed treatment-related pale kidneys in males at 4000 ppm. Immunotoxicological investigations revealed no treatment-related effects on cell counts in the spleen and the plaque forming cells assay (PFCA) revealed

no treatment-related effects. Flow cytometric evaluation after treatment revealed a treatment related decrease of $CD45^{total}$ and $CD45^{high}$ positive spleen cells and an increase of $CD45^{low}$ positive spleen cells in both sexes at 4000 ppm. A treatment-related decrease of the antibody titer of IgA at 4000 ppm in females was observed. **No adverse effects.** NOEL (M) = 33.60 mg/kg/day (400 ppm) and (F) = 4.00 mg/kg/day (40 ppm) (based on increased mean relative liver weights and decrease in CD45 lymphocytes). **Acceptable**. (Corlett, 06/27/07)